In the Specification:

Please amend the specification as shown:

Please delete the paragraph on page 10, lines 18-22, and replace it with the following paragraph:

Fig. 3B **(SEQ ID NOS 1-8, respectively in order of appearance)** shows the sequence of the different RNAi molecules used in the experiment the result of which is depicted in Fig. 3A. Fig. 3C shows the result of an immunoblot analysis of PTEN protein after treatment with modified RNAi molecules in comparison to PTEN specific antisense constructs.

Please delete the paragraph on page 10, lines 22-25, and replace it with the following paragraph:

Fig. 4 shows that the 3' overhang of RNAi molecules is not important for RNA interference. More particularly, Fig. 4A shows a dose response curve of different RNAi molecules and Fig. 4B **(SEQ ID NOS 9-20, respectively in order of appearance)** shows the sequence of the RNAi molecules used in the experiment the result of which is shown in Fig. 4A.

Please delete the paragraph on page 10, lines 26-29, and replace it with the following paragraph:

Fig. 5 shows that duplex length of the RNAi molecules has to be at least 18-19 nucleotides. More particularly, Fig. 5B **(SEQ ID NOS 21-28, respectively in order of appearance)** shows the sequence of the PTEN specific RNAi molecules used in the experiment the result of which is depicted in Fig. 5A as dose response curve.

Please delete the paragraph on page 10, lines 30-33, and replace it with the following paragraph:

Fig. 6 shows that four terminal mismatched nucleotides in RNAi molecules with a length of 19 nucleotides are still functional in mediating Akt1 knockdown. More particularly, Fig. 6B **(SEQ ID NOS 29-36, respectively in order of appearance)** shows the sequence of the RNAi molecules used in the experiment the result of which is depicted in Fig. 6A.

Please delete the paragraph on page 11, lines 1-12, and replace it with the following paragraph:

Fig. 7 shows further results on duplex length requirements and tolerance for mutation in siRNAs. More particularly, Fig. 7A **(SEQ ID NOS 37-52, respectively in order of appearance)** shows the various constructs used (left panel) and the respective impact on inhibition of Akt1 mRNA expression in HeLa cells relative to the expression of p110α used in the indicated amounts of siRNA molecules (right panel). The nucleotide changes in the mismatch siRNA molecules are indicated by arrows; the 3' desoxynucleotides, if any, are indicated in capital letters. Fig. 7B **(SEQ ID NOS 53-62, respectively in order of appearance)** shows the various PTEN specific siRNAs (left panel), the inhibition of PTEN mRNA expression in HeLA cells expressed as ratio PTEN/p110α, at various amounts of siRNA (middle panel) and Fig. 7C a Western Blot analysis depicting the inhibition of PTEN protein expression using PTEN specific siRNA (30nM) and respective mismatch siRNA after 48 and 96 hours, respectively, with p100α being used as loading control.

Please delete the paragraph on page 11, lines 13-17, and replace it with the following paragraph:

Fig. 8 shows the result of studies on the stability in serum conferred to RNAi molecules by 2'-O-methylation and that end modifications have no beneficial effects on RNAi stability. More particularly, Fig. 8A shows the result of a gel electrophoresis of the various RNAi molecules depicted in Fig. 8B **(SEQ ID NOS 9, 10, 63-68, 13, 14, 17 and 69-71, respectively in order of appearance)** being subject to incubation with fetal calf serum.

Please delete the paragraph on page 11, lines 18-24, and replace it with the following paragraph:

Fig. 9 shows that an amino end modification results in loss of activity. Fig. 9B **(SEQ ID NOS 72, 77, 73, 77, 74, 77, 75, 77, 78, 77, 76, 77, 80, 77, 78 and 79, respectively in order of appearance)** shows the particular RNAi molecules used in the experiments the result of which is shown in Fig. 9A expressed as PTEN/p110α expression level ratio. Fig. 9C shows the design principles which may be deduced from the results depicted in Fig. 9A. As used in Fig. 9C the term functional means functionally active in the particular assay system as described in the example and "not functional" means not functionally active in said system.

Please delete the paragraph on page 11, lines 25-30, and replace it with the following paragraph:

Fig. 10 shows that 2'-O-Alkyl (methyl) modifications stabilize RNAi molecules but also result in reduction of their activity. More particularly, Fig. 10C shows the sequence of the RNAi molecules used in the experiment the result of which is depicted as a dose response curve in Fig. 10A. Fig. 10B shows the result of a gel electrophoresis of the various RNAi molecules depicted in Fig. 10C **(SEQ ID NOS 13, 14, 81-90, 80, 90, 89, 14, 87 and 90, respectively in order of appearance)** being subject to a two hour incubation in fetal calf serum.

Please delete the paragraph on page 11, line 31, through page 12, line 3, and replace it with the following paragraph:

Fig. 11 shows the result of an experiment on the efficacy of RNAi molecules with blocks of 2'-O-methyl modifications with Fig. 11A graphically depicting the results of said experiments as a dose response curve and with Fig. 11C showing the sequences of the particular RNAi molecules used in said experiments. Fig. 11B shows the result of a gel electrophoresis of the various RNAi molecules depicted in Fig. 11C **(SEQ ID NOS 91-97, 90, 98-100 and 90, respectively in order of appearance)** being subject to a two hour incubation in fetal calf serum.

Please delete the paragraph on page 12, lines 4-12, and replace it with the following paragraph:

Fig. 12 shows that alternating 2'-O-methyl modification result in activity of the modified RNAi molecules compared to unmodified forms. More particularly, Fig. 12B **(SEQ ID NOS 101-1118, respectively in order of appearance)** shows the sequence of the RNAi molecules used in this experiment the result of which is depicted in Fig. 12A. Fig. 12C shows the stability of said RNAi molecules following incubation in serum for two hours, whereas Fig. 12D shows an immunoblot for PTEN protein upon application of different RNAi molecules to HeLa cells. As may be taken therefrom RNAi molecules with alternating modifications are stabilized against endonuclease degradation and active in mediating a PTEN protein knock down.

Please delete the paragraph on page 12, line 24, through page 13, line 8, and replace it with the following paragraph:

Fig. 15 shows that siRNA molecules with distinct 2'-O-methyl ribonucleotides modifications show increased stability in serum and mediate protein knock-down in HeLa cells. More particularly, Fig. 15A **(SEQ ID NOS 119-146, respectively in order of appearance)** indicates the various siRNA molecule constructs used (left panel), whereby 2'-O-methyl ribonucleotides modifications are underlined and indicated by bold letters in the sequence. Inhibition of PTEN mRNA expression in HeLa cells transfected with the indicated amounts of modified siRNA molecules is expressed as ratio PTEN/p110α and indicated on the right panel. Fig. 15B **(SEQ ID NOS 119, 120, 123, 124, 135-138, 141, 142, 147-152, 145 and 146, respectively in order of appearance)** shows on the left panel the various siRNA constructs used and on the right panel a PAA gel electrophoresis of modified and unmodified siRNA molecules after incubation in serum; the various constructs with 2'-O-methyl ribonucleotides are indicated by underlining and bold printing. Fig. 15C shows an SDS-PAGE based immunoblot illustrating the inhibition of PTEN protein expression using various of the siRNA constructs (30 nM) as depicted in Fig. 15A and 15B, respectively. Again, p110α is used as loading control. Finally, Fig. 15D is an immunoblot indicating a prolonged protein knock-down, i.e. the inhibition of PTEN protein expression, upon

administration of siRNA molecules (30 nM) with distinct 2'-O-methylribonucleotides modifications after 48 and 128 hours. As in Fig. 15C, p 110α is used as loading control.

Please delete the paragraph on page 13, lines 9-22, and replace it with the following paragraph:

Fig. 16 shows that siRNA molecules with distinct 2'-O-methylribonucleotides modifications which are specific for Akt1 and p110β mRNA show increased stability in serum and mediate protein knock-down in HeLa cells. More particularly, Fig. 16A **(SEQ ID NOS 153-164, respectively in order of appearance)** indicates on the left panel the various constructs used whereby again 2'-O-methylribonucleotides are underlined and printed in bold. The integrity of the indicated siRNA molecules after incubation in serum is shown in the right panel. Fig. 16B shows an immunoblot of Akt1, Akt2 and Akt phosphorylation and p110 being used as a loading control upon transfection of the cells with the indicated siRNAs (30 mM). Fig. 16C **(SEQ ID NOS 165-174, respectively in order of appearance)** shows various p110β specific siRNA constructs (left panel) with the 2'-O-methyl modifications being underlined and printed in bold, and the result of an immunoblot analysis (right panel) of the inhibition of the phosphorylation of the downstream kinase Akt1 by said siRNA constructs. p110α has been used as a loading control.

Please delete the paragraph on page 13, line 23, through page 14, line 5, and replace it with the following paragraph:

Fig. 17 shows the efficacy of various RNAi molecules with hairpin structures as dose response curve while Fig. 17B shows the structure of the RNAi molecules the result of which is depicted in Fig. 17A. Synthetic siRNAs with different loops are functional in reducing the p110β , Akt1 and Akt 2 expression. (14**A**) Inhibition of p110β mRNA expression in siRNA transfected HeLa cells. Samples were analyzed in parallel for the level of p110β mRNA expression 24h after transfection of the indicated siRNAs. The transfected bimolecular siRNAs (21mer with 3' TT overhangs, molecule 1AB) or the monomolecular siRNAs with loop structures are schematically shown. Note that the position of the loops (HIV derived pA-loop; $(A)_{12}$-loop) **(SEQ ID NO: 175)** relative to the antisense sequence is reversed in 3A, 4A

relative to 3B, 4B. The 2AB siRNA molecule contains 6 mismatches in the 21mer duplex and serves as a negative control together with the untreated sample. RNA was prepared und subjected to real time RT-PCR (Taqman) analysis. p110β mRNA levels are shown relative to the mRNA levels of p110α, which serve as an internal reference. Each bar represents triplicate transfections (± standard deviation). HeLa cells were transfected at 50% confluency (2500 cells per 96 well) with siRNAs at the indicated concentrations in growth medium.

Please delete the paragraph on page 14, lines 6-18, and replace it with the following paragraph:

Fig. 18 shows the efficacy of various RNAi molecules with intermolecular and intramolecular loop structures as dose response curves. (18**A**) **(SEQ ID NO: 175)** Inhibition of Akt1 mRNA expression in siRNA transfected HeLa cells. Samples were analysed in parallel for the level of Akt1 and Akt2 mRNA expression 24h after transfection of the indicated siRNAs. The different loops (A-loops; GAGA-loop and a polyethyleneglycol (PEG)-linker) and their putative secondary structure are shown schematically. The siRNA molecule 9A is specific for Akt2 and serves as a negative control. Note that 10A and 10B do not contain self-complementary sequences and are transfected in combination. Akt1 mRNA levels is shown relative to the mRNA levels of p110β , which served as internal control. (**18B**) **(SEQ ID NO: 175)** Inhibition of Akt2 mRNA expression in HeLa cells transfected with the indicated siRNA molecules. Akt2 mRNA levels is shown relative to the mRNA levels of p110β. The Akt1 specific molecule 7A serves here as a negative control.

Please delete the paragraph on page 32, lines 4-22, and replace it with the following paragraph:

Determination of the relative amounts of RNA levels by Taqman analysis:

24h post transfection the RNA of cells transfected in 96-wells was isolated and purified using the Invisorb RNA HTS 96 kit (InVitek GmbH, Berlin). Inhibition of PTEN mRNA expression was detected by real time RT-PCR (Taqman) analysis using 300 nM PTEN 5' primer CACCGCCAAATTTAACTGCAGA **(SEQ ID NO: 176)**, 300 nM PTEN 3'

primer AAGGGTTTGATAAGTTCTAGCTGT **(SEQ ID NO: 177)** and 100 nM of the PTEN Taqman probe Fam-TGCACAGTATCCTTTTGAAGACCATAACCCA-Tamra **(SEQ ID NO: 178)** in combination with 40 nM β-actin 5' primer GTTTGAGACCTTCAACACCCCA **(SEQ ID NO: 179)** , 40 nM β-actin 3' primer GACCAGAGGCATACAGGGACA **(SEQ ID NO: 180)** and 100 nM of the β-actin Taqman probe Vic-CCATGTACGTAGCCATCCAGGCTGTG-Tamra **(SEQ ID NO: 181)**. The Akt primers and probes are determined in Sternberger et al. (Sternberger, supra) and are used according to the manufacturer's instructions (Applied Biosystem; use of Amplicon Set). Also said primers and probes may be designed using the software program Primer Express (Applied Biosystem). The reaction was carried out in 50 µl and assayed on the ABI PRISM 7700 Sequence detector (Applied Biosystems) according to the manufacturer's instructions under the following conditions: 48°C for 30 min, 95°C for 10 min, followed by 40 cycles of 15 sec at 95°C and 1 min at 60°C.

Please delete the paragraph on page 33, lines 12-21, and replace it with the following paragraph:

Antibodies. The murine monoclonal anti-p110 antibody U3A and the murine monoclonal anti-p85 antibody N7B have been described (Klippel et al., 1994, supra). Rabbit polyclonal anti-Akt and anti-phospho Akt (S473) antibodies were obtained from Cell Signaling Technology. The murine monoclonal anti-PTEN antibody was from Santa Cruz Biotechnology. The PTEN 53 specific antisense molecule, *i.e.* geneBloc, is described in Sternberger *et al.* [Sternberger, *supra*] having the following sequence (ucuccuuTTGTTTCTGcuaacga) **(SEQ ID NO: 182)**, whereby the nucleotide depicted in lower case are ribonucleotides whereas the nucleotide in capital letters are desoxyribonucleotides. This antisense molecule is also identical to RNAi 1A without TT.

Please delete the paragraph on page 47, lines 3-10, and replace it with the following paragraph:

A dose dependent titration showed no significant difference in efficiency of mRNA knock-down achieved by the standard bimolecular double strand 21mer and the corresponding monomolecular molecules as analysed by realtime PCR (Taqman) (Fig.

17A). Two different loop structures a $(A)_{12}$ loop **(SEQ ID NO: 175)** and a HIV derived pA-loop were tested in parallel with similar results. A comparision of the relative position of the antisense sequence and the loop structure revealed an improved knock-down efficiency with the antisense sequence being located 3' to the loop (Fig. 17B; compare construct 3A, 3B and 4A, 4B).